

CLAIMS

1. A conjugate of a hapten to a natural or synthetic  $\beta$ -lactam derivative, wherein the  $\beta$ -lactam derivative is at least partially constitutive of the  
5 conjugating arm.

2. A conjugate according to Claim 1, wherein said conjugating arm is bound to the amine group located on the  $\beta$ -lactam nucleus.

3. A conjugate according to Claim 2, wherein  
10 the part of the conjugating arm located between the hapten moiety and the side chain of the  $\beta$ -lactam derivative includes a number of atoms comprised between 0 and 10.

4. A conjugate according to Claim 3, wherein the number of atoms is comprised between 0 and 4.

5. A conjugate according to Claim 3, wherein  
15 said atoms are carbon atoms and heteroatoms selected from the group consisting of oxygen, sulphur and nitrogen.

6. A conjugate according to Claim 1, wherein the hapten is selected from the group consisting of  
20 steroids, drugs, drugs of abuse and medicines.

7. A conjugate according to Claim 1, wherein said  $\beta$ -lactam derivative is selected from the group consisting of penicillins and cephalosporins.

8. A conjugate according to Claim 6 wherein  
25 the hapten is chosen from the group consisting of nandrolone, testosterone, progesterone, estradiol and cocaine.

9. A conjugate according to Claim 7, wherein the  $\beta$ -lactam derivative is selected from the group  
30 consisting of carbenicillin, oxacillin, cefuroxime, cefotaxime, methicillin, benzylpenicillin and phenoxymethylpenicillin.

10. A conjugate according to Claim 8 or 9 wherein said conjugate is selected from the group consisting of nandrolone carbenicillinate, cocaine carbenicillinate, progesterone oxacillinate and  
5 progesterone benzylpenicillinate.

11. Method for the immunoassay of a hapten involving a  $\beta$ -lactam derivative-based inhibitor-hapten conjugate according to Claim 1, wherein :

- said  $\beta$ -lactam derivative-based inhibitor-hapten  
10 conjugate binds, competitively with the free hapten to be assayed in a solution, to an anti-hapten antibody ;
- said conjugate, when unbound to said antibody, is capable to bind to a protein receptor site for the  $\beta$ -lactam moiety, the antibody-bound conjugate being unable to  
15 bind to said receptor, owing to steric hindrance ;
- the recognition of the conjugate binding to the receptor is associated to detection and/or quantification means.

12. Method according to Claim 11, wherein the recognition of the conjugate binding to the protein receptor  
20 is related to the modulation of an enzyme activity of said protein against a reporter substrate.

13. Method for the immunoassay in an homogeneous phase of a hapten involving a  $\beta$ -lactam derivative-based inhibitor-hapten conjugate according to  
25 Claim 11, comprising the steps of :

- adding a known quantity of inhibitor-hapten conjugate (IH<sub>b</sub>) to a sample solution containing the free hapten (H<sub>f</sub>) to be detected and/or quantified ;
- adding a quantity of antibody (AB) directed at the hapten  
30 in its free and conjugate state and related to the quantity of said conjugate in solution ;
- adding to the solution a  $\beta$ -lactamase having an active site for two substrates entering into competition on

said active site, the first substrate being a reporter substrate (S) transforming into a product detectable and/or quantifiable, the second substrate being the inhibitor-hapten conjugate (IH<sub>b</sub>) modulating the rate of hydrolysis of the reporter substrate (S), the higher the quantity of free hapten (H<sub>f</sub>) initially in solution, the lower the quantity of inhibitor-hapten conjugate having bound one said antibody (IH<sub>b</sub> - AB) and the higher the quantity of said conjugate binding at the active site (IH<sub>b</sub> - E) and the lower the enzymatic activity against the reporter substrate (S).

14. Method according to Claim 13, wherein the  $\beta$ -lactamase is a class C  $\beta$ -lactamase.

15. Method according to Claim 14, wherein the class C  $\beta$ -lactamase is selected from the group consisting of class C  $\beta$ -lactamases extracted from *Enterobacter cloacae*, *Escherichia coli* and *Citrobacter freundii*.

16. Method for the immunoassay of a hapten involving a  $\beta$ -lactam derivative-based inhibitor-hapten conjugate according to Claim 11, comprising the steps of :

- adding a known quantity of inhibitor-hapten conjugate (IH<sub>b</sub>) to a sample solution containing the free hapten (H) to be detected and/or quantified ;
- adding a quantity of antibody (AB) directed at the hapten in its free and conjugate state and corresponding to the quantity of said conjugate in solution ;
- adding to the solution a penicillin detector (D) capable of specific recognition of the  $\beta$ -lactamic moiety of said conjugate (IH<sub>b</sub>), the bound quantity of said conjugate (IH<sub>b</sub>) to said detector (D) being modulated in a competitive reaction of the free hapten (H<sub>f</sub>) initially in solution and said conjugate (IH<sub>b</sub>) with said antibody (AB).

17. Method according to Claim 16, wherein the penicillin detector is a polypeptide included in a detection kit selected from the group consisting of BetaSTAR<sup>®</sup>, SNAP<sup>®</sup> Beta Lactam, Penzym<sup>®</sup>, Parallux<sup>®</sup> Beta Lactam assay, Charm Farm Test<sup>®</sup>, Delvo-X-press<sup>®</sup>, Delvotest<sup>®</sup> P and Delvo<sup>®</sup> test SP.

18. Method according to Claim 12, wherein the reporter substrate is nitrocefin.

19. Method according to Claim 12, wherein the reporter substrate is cephalixin.

20. Method according to Claim 11 or 16, wherein substances are added to the hapten-containing solution to be assayed for removing possible interference.

21. Method according to Claim 20, wherein said substances are selected from the group consisting of agents for protecting  $\beta$ -lactamase and penicillin detector, agents for protecting reporter substrate, agents for protecting hapten-inhibitor complex and decontaminating agents.

22. Method according to Claim 21, wherein said substances are selected from the group consisting of class B  $\beta$ -lactamases, sodium azide and phenylbutazone.

23. Method according to Claim 22, wherein the class B  $\beta$ -lactamase is extracted from *Bacillus cereus*.

24. Method according to Claim 12, wherein a starch-iodine color system is used for revealing the hydrolysis rate of the reporter substrate, iodine being generated in a solution of starch paste stabilized by addition of cadmium iodide.

25. Method according to Claim 24, wherein the iodine is generated from cadmium iodide in the presence of a complexing agent and an oxidizing agent in a medium of

appropriate pH and then bringing the revealing reagent to the working pH.

26. Method according to Claim 25, wherein the complexing agent used is DTPA.

5 27. Method according to Claim 25, wherein the oxidizing agent is an iodate or a periodate.

28. A kit of reagents for the immunoassay of a hapten, comprising :

- a buffer liquid for immunoassay containing a known  
10 quantity of  $\beta$ -lactam derivative-based inhibitor-hapten conjugate, said  $\beta$ -lactam derivative being selected from the group consisting of carbenicillin, oxacillin, cefuroxime, cefotaxime, methicillin, benzylpenicillin and phenoxymethylpenicillin ;
- 15 - a quantity of antibody directed at the hapten in its free and conjugate state and related to the quantity of inhibitor-hapten conjugate in solution ;
- a  $\beta$ -lactamase enzyme associated with a known quantity of reporter substrate to be hydrolyzed by the enzyme or a  
20 penicillin detector capable of specific recognition of the  $\beta$ -lactamic moiety of said conjugate ;
- reagents for detecting and/or quantifying enzyme or penicillin detector activity.

29. A kit of reagents according to Claim 28,  
25 comprising at least one reagent selected from the group consisting of agents for protecting  $\beta$ -lactamase, agents for protecting reporter substrate, agents for protecting hapten-inhibitor complex and decontaminating agents.